1787-Pos Board B697
Effects of Glycerol and Urea on Micellization, Membrane Partitioning and Solubilization by a Non-Ionic Surfactant
Hiren Patel, **Gaurav Raval**, Mozhgan Nazari, Heiko Heerklotz.
We have studied the effect of two cosolvents, urea and glycerol, on the association and interactions of a surfactant, octaethyleneglycol dodecyl ether (C12EO8) and a phospholipid (POPC). We have measured the CMC, the partition coefficient, the effective mole fractions Xesat and Xesol at the onset and completion of the membrane-to-micelle transition (membrane solubilization), and the enthalpies of transfer of surfactant by ITC. Changes in membrane order and dynamics were characterized by time-resolved fluorescence anisotropy measurements of DPH, and micelle sizes and clouding by light scattering. The cosolvents have complex effects that are not governed by the well-known 'salting in' or 'salting out' effects on the solubility alone. Instead, urea and glycerol alter also the intrinsic curvature ('effective molecular shape') of the detergent and the order and packing of the membrane. These curvature effects have an unusual enthalpy/entropy balance and are not additive for lipid and detergent. The results shed light on the phenomena governing lipid-detergent systems in general and have a number of implications for the use of cosolvents in membrane protein studies.

1788-Pos Board B698
Attack or Retreat: Two Modes of Membrane Solubilisation by Surfactants
Mozhgan Nazari, Heiko Heerklotz.
Solubilization of a lipid membrane by a micelle-forming surfactant starts with the appearance of mixed micelles coexisting with mixed membranes and aqueous phase. This occurs at a characteristic membrane composition (often given as the mole ratio Resat) and a characteristic aqueous concentration of surfactant that is typically somewhat below the CMC of the pure surfactant. Thermodynamically, both criteria are fully equivalent because they linked by the partition coefficient. However, there are two scenarios when it comes to the mechanism of solubilization. On one hand, the surfactant may destroy the membrane by inducing a critical curvature strain superseding the mechanical stability of the bilayer. On the other hand, the surfactant may de-mix from the membrane and associate in the aqueous phase to form micelles that extract lipid from the membrane. Time-resolved fluorescence anisotropy data of DPH derivatives agree with other parameters reflecting membrane order in that there is a characteristic, minimum order of a given membrane (at a certain temperature) and most detergents have to disorder the membrane to this critical state before micelles appear and solubilization proceeds. However few, mainly bio- and bioanalogous surfactants were found to solubilize by demixing without critical disordering of the membrane. This may account for the superior performance of the latter in solubilizing membrane proteins in their active state.

1789-Pos Board B699
Interactions of Dodecylphosphocholine with Lipid Membranes
Arpit Shah, Hiren Patel, Mozhgan Nazari, Heiko Heerklotz.
As a bio-analogous surfactant, n-dodecylphopsphocholine (abbreviated DPC, LPC, or FOS-choline-12) has been proven useful for membrane protein studies. We present a detailed characterization of its temperature-dependent self-association (CMC, deltaCp) and its interaction with liposomes. We describe membrane partitioning, membrane permeation, membrane permeabilization (i.e., leakage to aqueous solutes), membrane lysis/solubilization to micelles and structural aspects by means of ITC (demicellization, uptake-and-release, solubilization-and-reconstitution assays), static and dynamic light scattering (NIBS), the lifetime-based dye leakage assay (for details on the method see Soft Matter 2009, 5:2849), and the time-resolved fluorescence anisotropy of membrane probes. The results are important for the optimization of membrane protein solubilization and reconstitution into proteoliposomes.

1790-Pos Board B700
Reproduction of Vesicles upon Fatty Acid Addition
Nicole A. Pfleger, Albert J. Markvoort, Ben de Kruijff, Rutger A. van Santen, J. Antoinette Killian.
A pre-requisite for life is the formation of membranes. Those membranes need to be stable under a wide range of conditions, but still need to be able to organize into different supramolecular structures to allow them to respond to changes in the environment. One of the challenges the membranes need to face is to deal with all the changes (e.g. size/ shape/ aggregation forms) that are required for the reproduction of cell like systems.
Previously [1] we suggested a mechanism of the reproduction of lipid vesicles upon fatty acid addition. One important feature for this mechanism is the relative rate of two processes: The insertion of material into the outer leaflet on the one hand and the equilibration of the material concentration gradient across both leaflets on the other hand. Based on a combination of experiments and molecular dynamic simulations we proposed a mechanism of ''vesicle replication by growth and division'' with the interesting property that the contents of the vesicles do not leak out during this process.
Here we provide additional support for this mechanism based on density gradient separation of vesicles and on experiments with different types of preformed seed vesicles. In addition these results indicate that the alternative mechanism of ''de novo'' formation as proposed for fatty acid seed vesicles [2] is highly unlikely for the reproduction of lipid containing vesicles.
[1] Markvoort *et al.* Biophys. J.(2010), 99, 1520-1528[2] Chen *et al.* Biophys. J.(2004), 87, 988-998

1791-Pos Board B701
The Competitive Binding of Ca^{2+} vs. Mg^{2+} to PIP2-Containing Lipid Monolayers and the Comparison of Pi(4,5)P2 and its Isomers
Yu-Hsiu Wang, Paul A. Janmey.
Interactions between divalent cations and PtdIns(4,5)2-containing lipid model membrane are investigated. Surface pressure measurements of lipid monolayers formed by binary lipid mixtures containing 25mol% PIP2 were used to quantify divalent cation binding. Direct titration and competitive binding assays show that divalent cations including Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and some multivalent polyamines bind PIP2 with K_d ranges from 200nM-50uM. Some of these cations, which are considered physiologically important, were examined more closely and evidence for divalent cation-induced lateral segregation of PIP2 is presented. Such studies are based on fluorescence and atomic force microscopic studies of Langmuir-Schaeffer lipid monolayers, and are coupled with numerical studies. Ca^{2+} and Mg^{2+} have similar binding affinity to PIP2-containing monolayers, but Ca^{2+} induces PIP2 lateral segregation more efficiently than compared to Mg^{2+}. Ca^{2+} vs. Mg^{2+} competitive binding assays are also carried out on PtdIns(3,4)P2- and PtdIns(3,5)P2-containing monolayers. Significant differences in the response of these three PIP2 isomers to divalent cations are found.
Besides monolayer studies, PIP2-cation interactions are also examined on a bilayer system. Fluorescence correlation spectroscopy (FCS) and laser scanning confocal microscopy (LSCM) are used following divalent cation titration on asymmetric labeled PIP2-containing giant unilamellar vesicles (GUVs). The diffusion coefficient and the fluorescence intensity variance of fluorescent PIP2 change with increasing divalent cation concentration. Förster resonance energy transfer (FRET) studies using bi-color labeled PIP2 are also carried out in a large unilamellar vesicle (LUV) system. These results together reveal that the lateral inhomogeneity of PIP2 changes with divalent cation concentration on bilayer model membranes. These results may provide insight into divalent cation-induced PIP2 microdomain formation in the cell membrane.

1792-Pos Board B702
Phosphatidyl Serine Containing Liposomes on Titania: Phase Behaviour, Bilayer Formation, and Lipid Asymmetry
Sandra Camarero, Cornelis Lütgebaucks, Rik Matena, Marta Gallego, Hanna Wacklin, **Ilya Reviakine**.
Interactions between surfaces of inorganic materials and biological systems are important in numerous technological contexts (implant integration, biosensor development). They also present basic challenges. For example, the role of surface ion equilibrium in the biological response to the material is not well understood, although a casual link between the two has been proposed. Here, we investigate the behaviour of phosphatidyl serine (PS)-containing liposomes on TiO2 as a function of liposome PS contents and solution Ca2+ concentration. We determine a ''phase diagram'', where a percolation-type transition between adsorbed liposomes and supported bilayers is observed, describe the driving force for this transition, and identify the role of surface heterogeneities in this porcess. Finally, we quantify the distribution of PS in the resulting supported bilayers by neutron reflectometry.

1793-Pos Board B703
Cholesterol Superlattice Modulates Drug Release from Liposomes
Berenice Venegas, Nicole B. Haloupek, Janet Lee, Elizabeth Zellhart, Mohammad F. Kiani, Parkson L.-G. Chong.
Combretastatin A4 disodium phosphate (CA4P) is an anticancer drug currently under Phase II clinical trials. CA4P disrupts tumor blood vessels, causing extensive tumor necrosis. Liposomal CA4P, as opposed to free CA4P, can reduce the drug's side effects and achieve targeted delivery. Previously we have shown that liposomal CA4P significantly delayed tumor growth in mice (Pattillo et al. (2009) Pharmaceutical Research 26, 1093-1100). However, the overall efficacy of the liposomal CA4P was still low. One of the strategies to improve this efficacy is to optimize the liposomal formulation. In this study, we have developed a new fluorescence assay and used it to examine the effect of cholesterol content on the release of entrapped CA4P from unilamellar vesicles (~180 nm) composed of cholesterol and POPC. We have used small cholesterol increments (0.5 mol%) over a wide range of cholesterol mole fractions